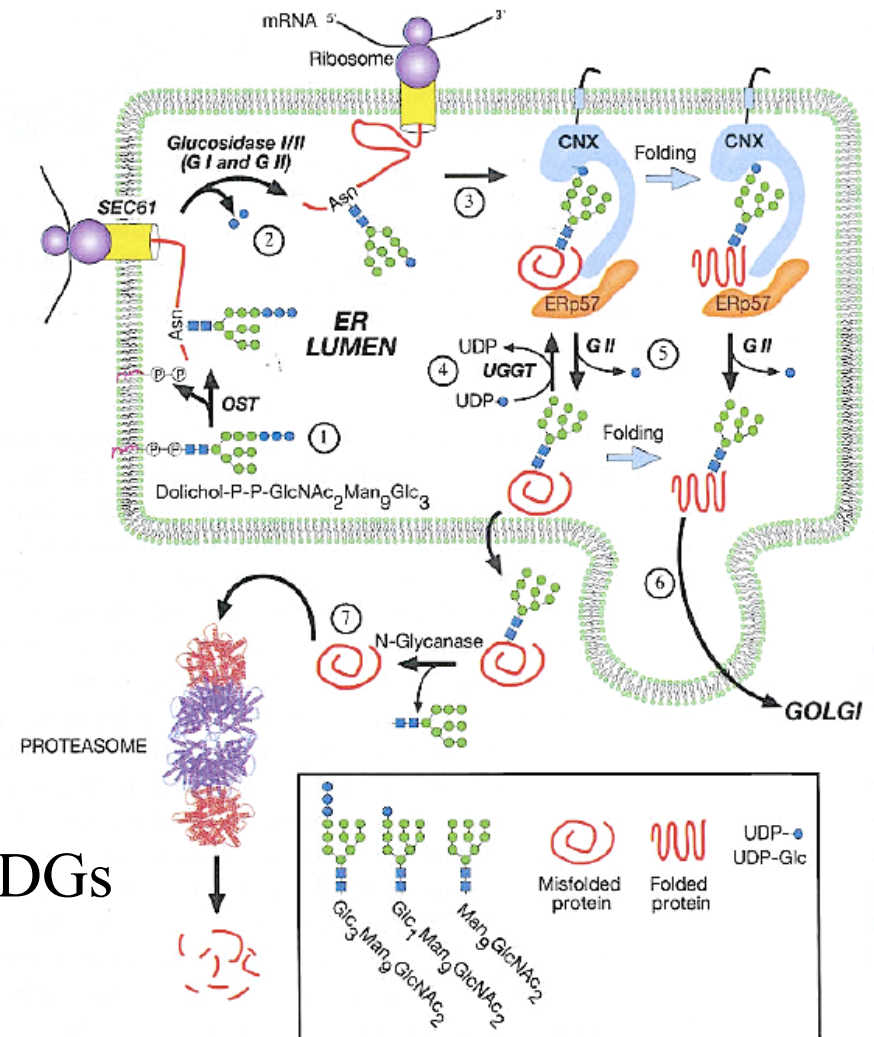
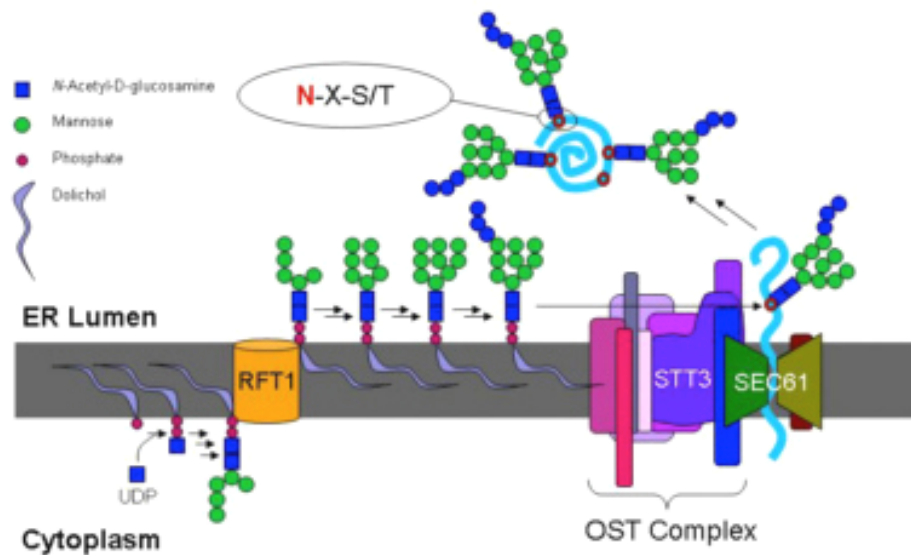


# Glycoprotein Diseases: Important, Unrecognized and Challenging



- N-linked Glycoprotein Biogenesis and CDGs
- N-linked Glycans and Quality Control
- NGLY1 Deficiency
- Clinical Features of NGLY1 Deficiency (Lynne Wolfe)
- Mannose Oligosaccharide Glucosidase Deficiency (Sergio Rosenzweig)

# NIH Glycosciences: A rich and lasting heritage



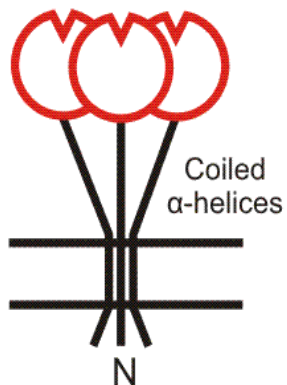
**Claude Hudson**

The founder of basic  
carbohydrate research  
at the NIH (Chief-1952)



**Hewitt G. Fletcher**

Chief, 1951-1973



**G. Gilbert Ashwell**

Discovery of Mammalian Lectins  
Chief, LBM, 1978-1983



**Elizabeth Neufeld**

Chief, GBB 1979-1983



**Victor Ginsburg**

Chief, Lab Structural Biology  
1986-1991



**Roscoe Brady**

NINDS  
1972 to 2006

Glycoscience Interest Group  
Undiagnosed Disease Program



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# Kids who don't cry: New genetic disorder discovered

By **Jacque Wilson**, CNN

🕒 Updated 2:53 PM ET, Thu March 20, 2014



Grace Wilsey was born with NGLY1 deficiency, which is caused by two mutations in the NGLY1 gene.

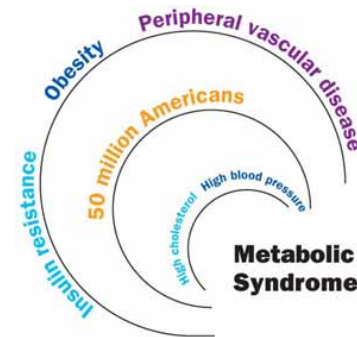
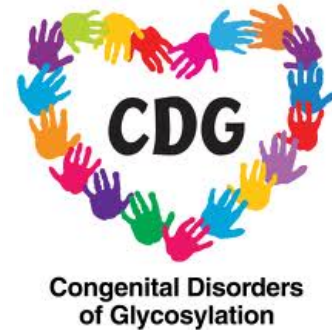


# Glycans play a major role in human disease:

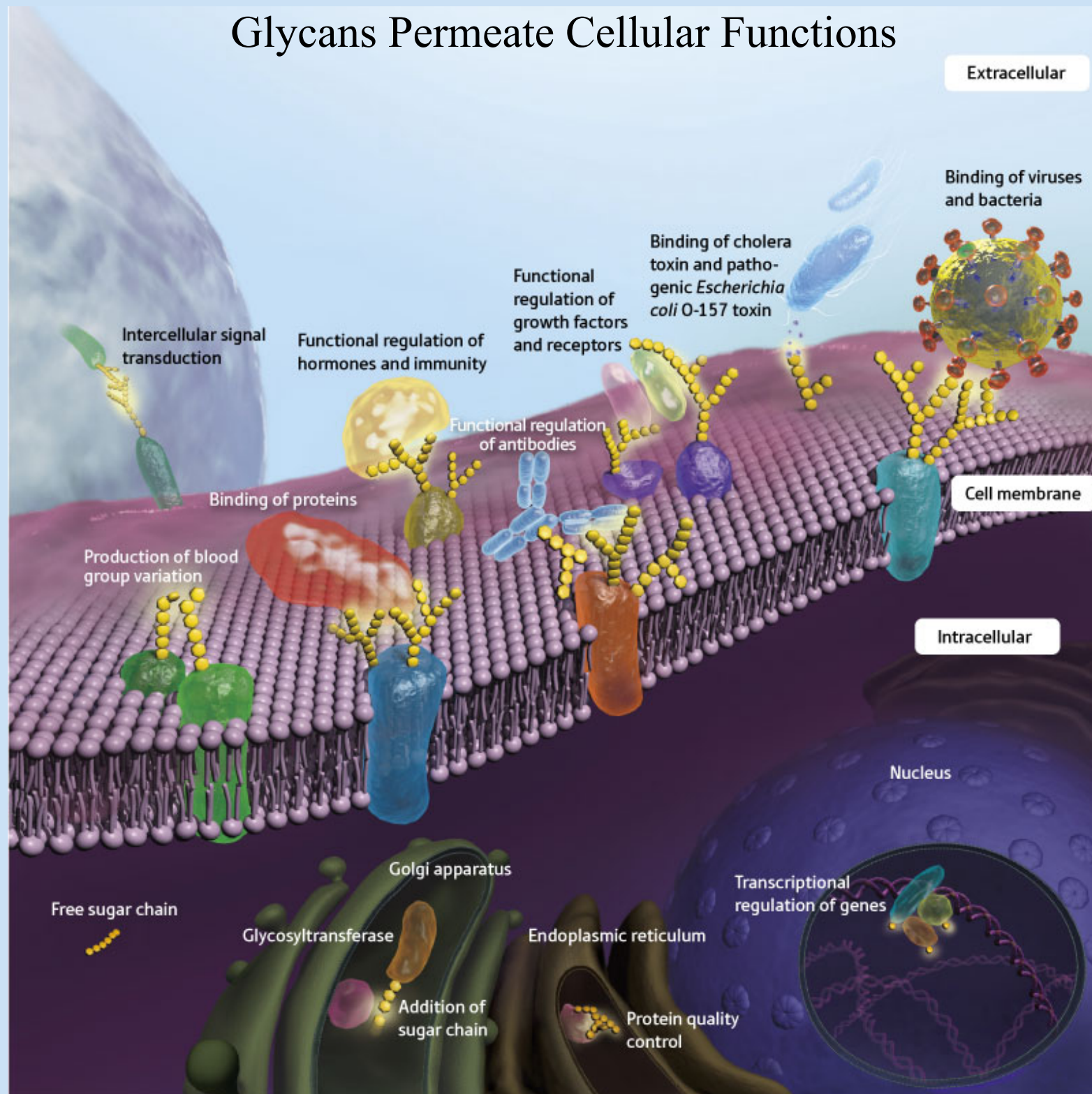
➤ **Rarity/Severity of genetic diseases highlight importance of glycans**

➤ **Some Examples of Glycans and Disease:**

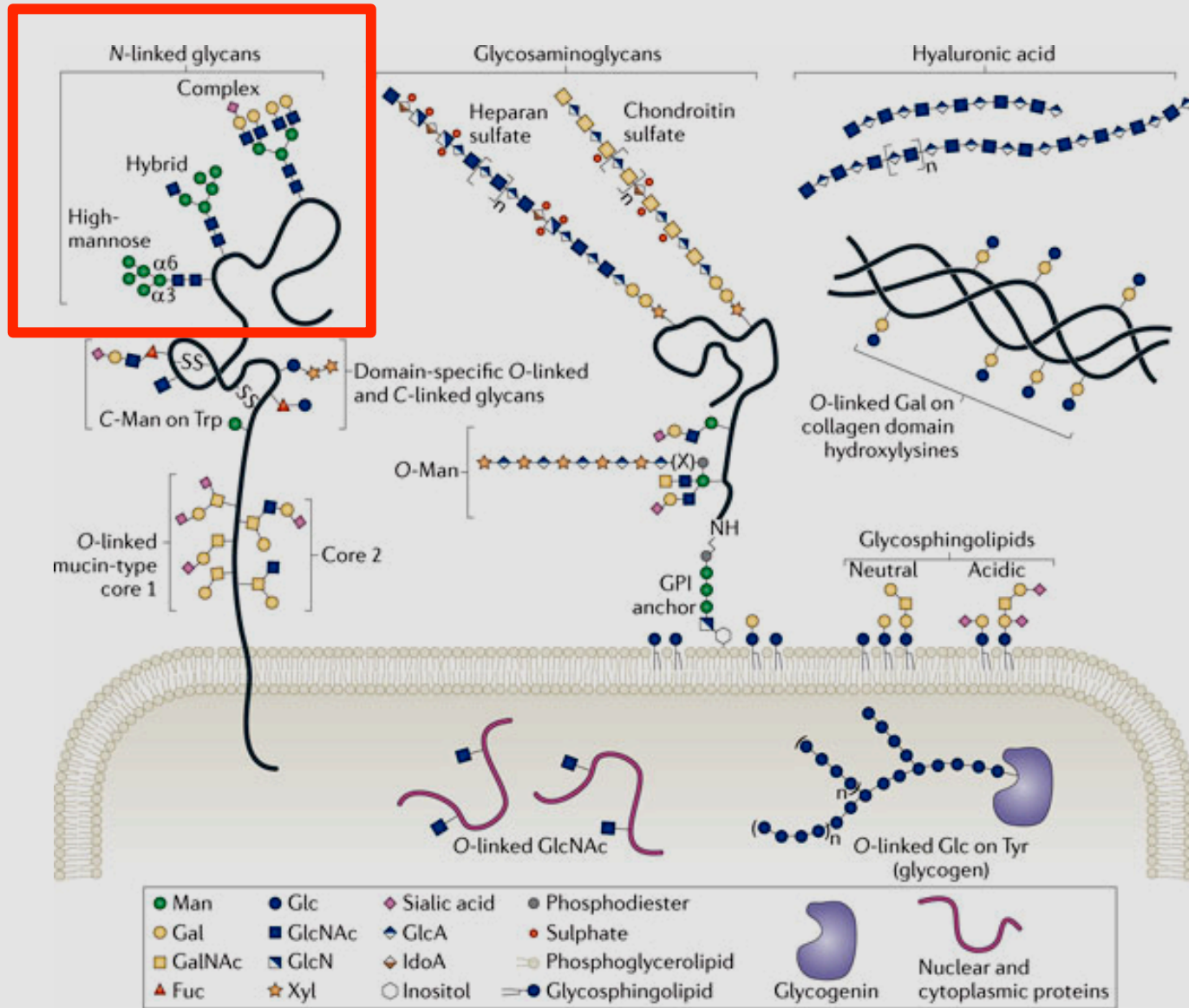
- \$ **Defective O-glycosylation in Muscular Dystrophy**
- \$ **O-GlcNAcylation: Diabetes, Alzheimer's, Cancer, Heart Disease.**
- \$ **Notch Signaling by Glycans**
- \$ **Selectins and Inflammation**
- \$ **Siglecs and Regulation of Immunity**
- \$ **Galectins role in immunity**
- \$ **Proteoglycans: growth factors, microbe binding, morphogenesis**
- \$ **Microbes and Viruses: Glycans role in entry and defense**
- \$ **Heparin – this 'drug' is a GAG.**
- \$ **Monoclonal Therapeutics – Glycoforms**
- \$ **Cell Surface Glycans in Tumor Metastasis – Cancer Biomarkers.**
- \$ **Vaccines to Infectious Organisms – Many (Most) are glycans.**



# Glycans Permeate Cellular Functions

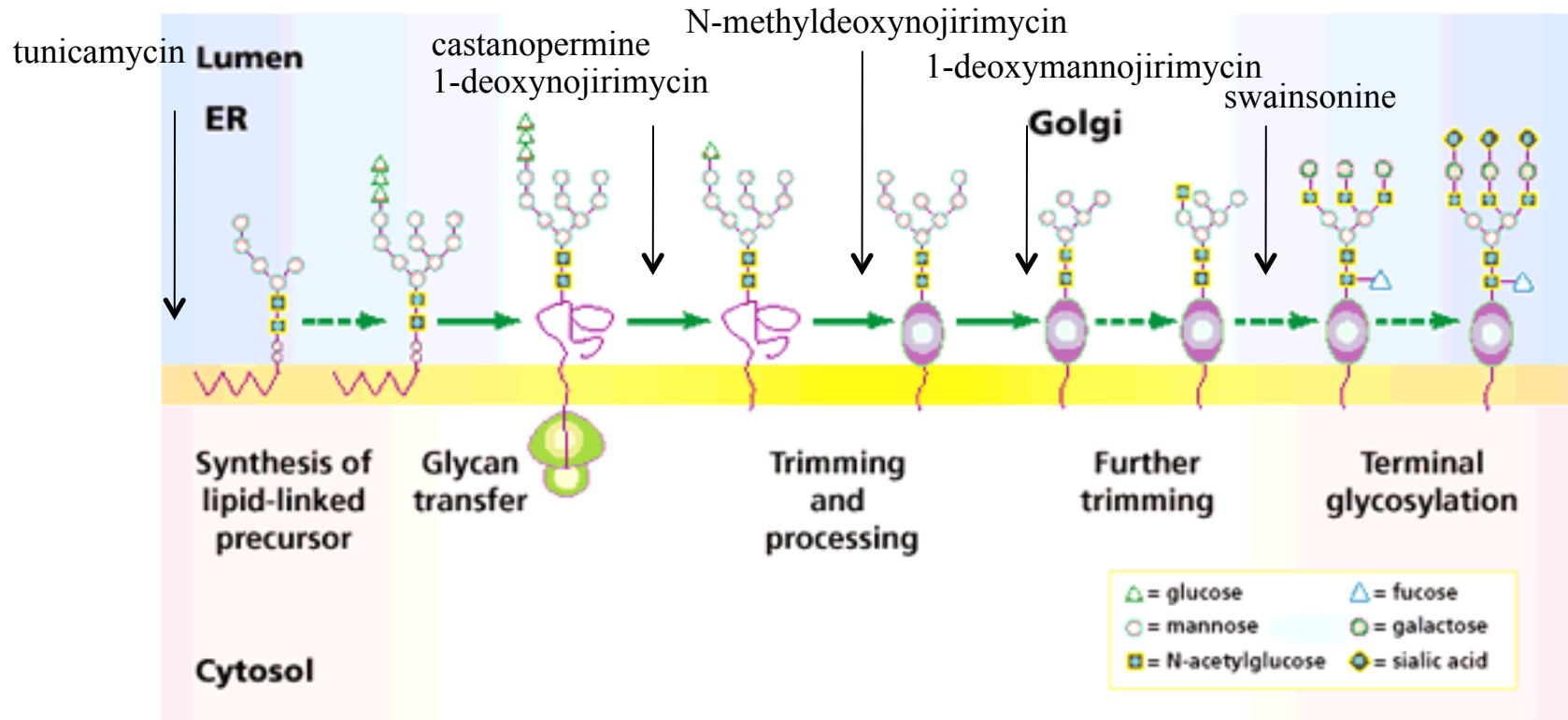


# Mammalian Glycoconjugates



# N-Glycan Biosynthetic Pathway: A System to Generate Diversity.

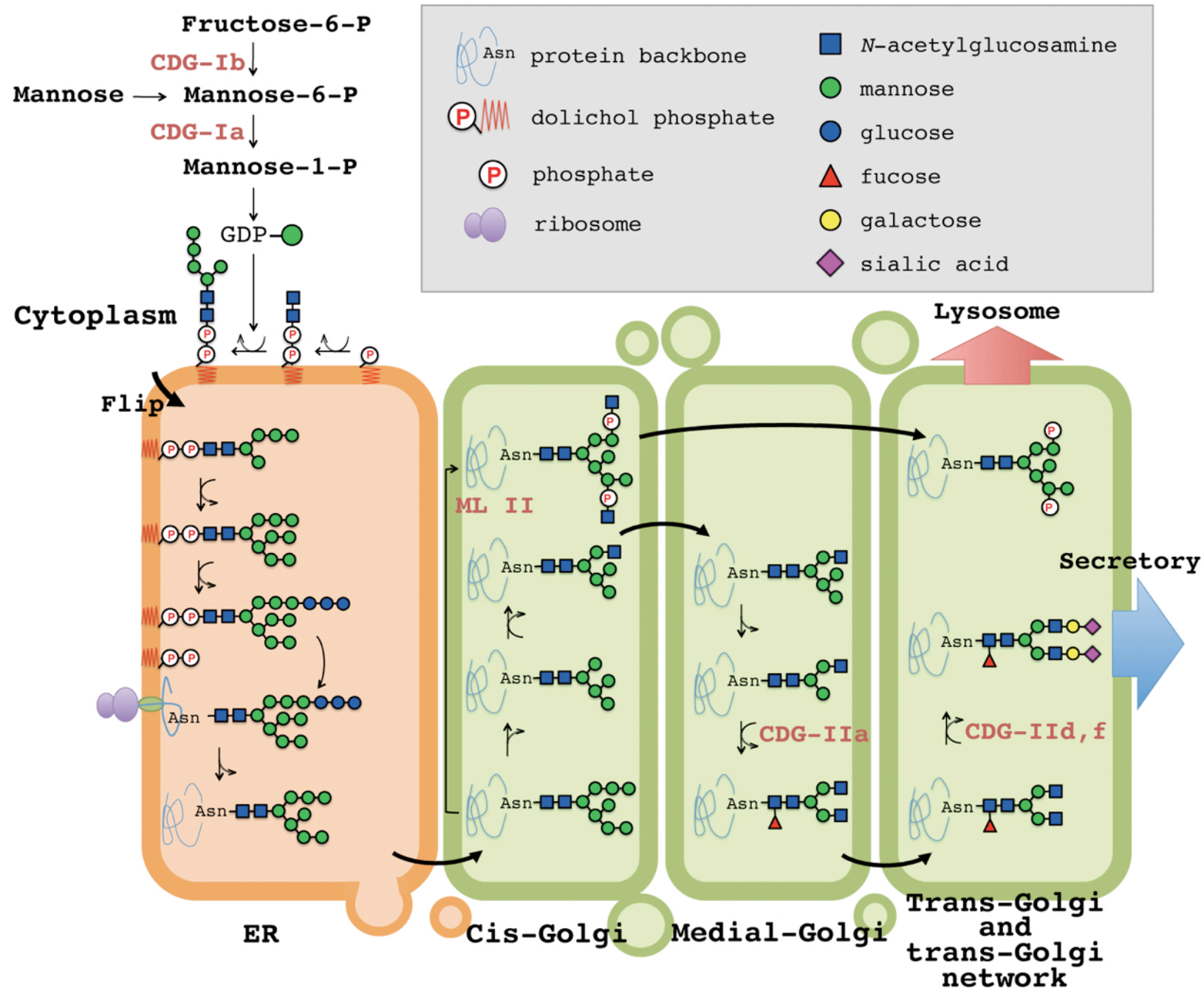
What do we know?



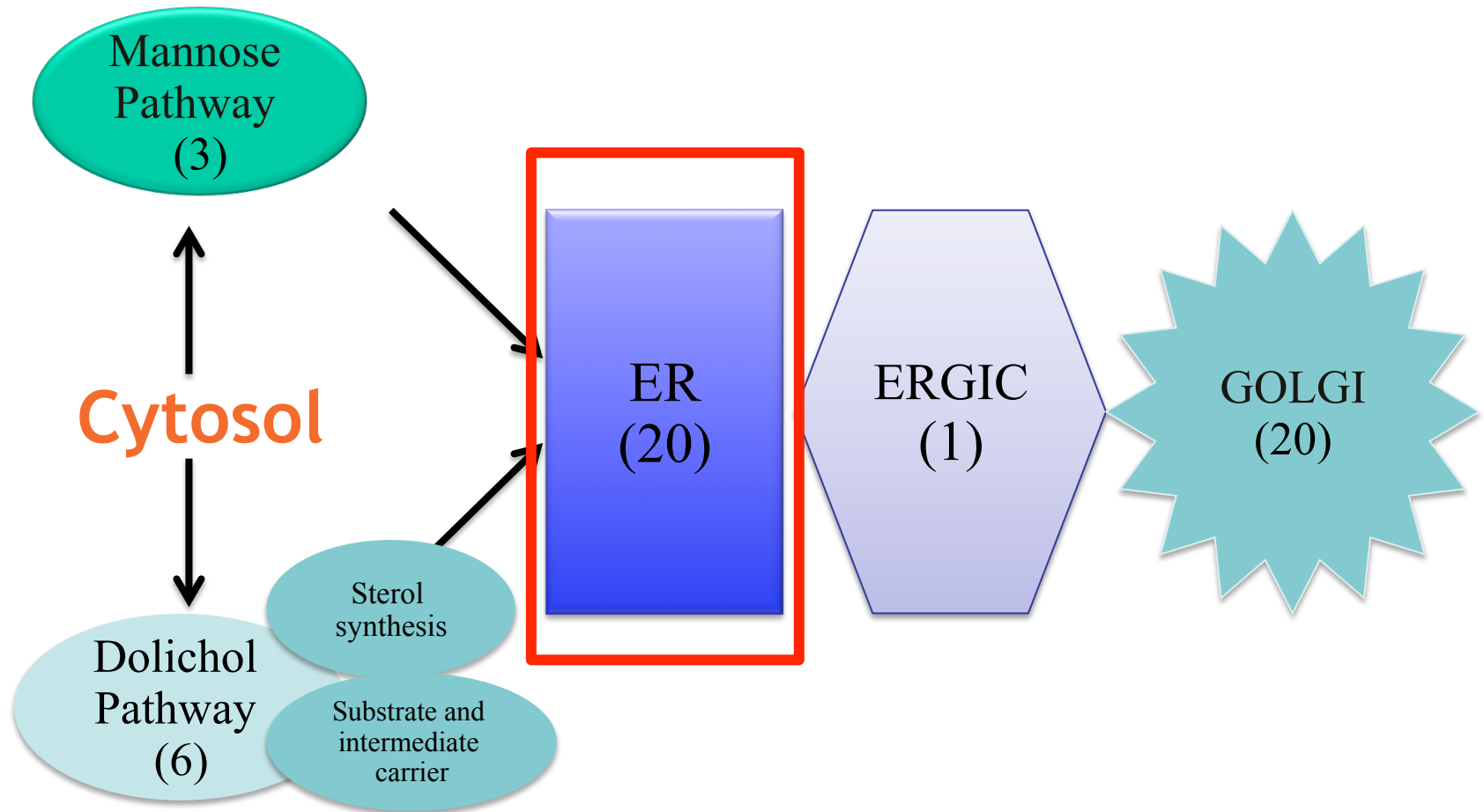
How did we learn it?

1. Biochemistry
2. Inhibitors
3. Yeast and Somatic Cell genetics
4. Congenital Disorders of Glycosylation

# Glycoprotein Biogenesis and the Congenital Disorders of Glycosylation

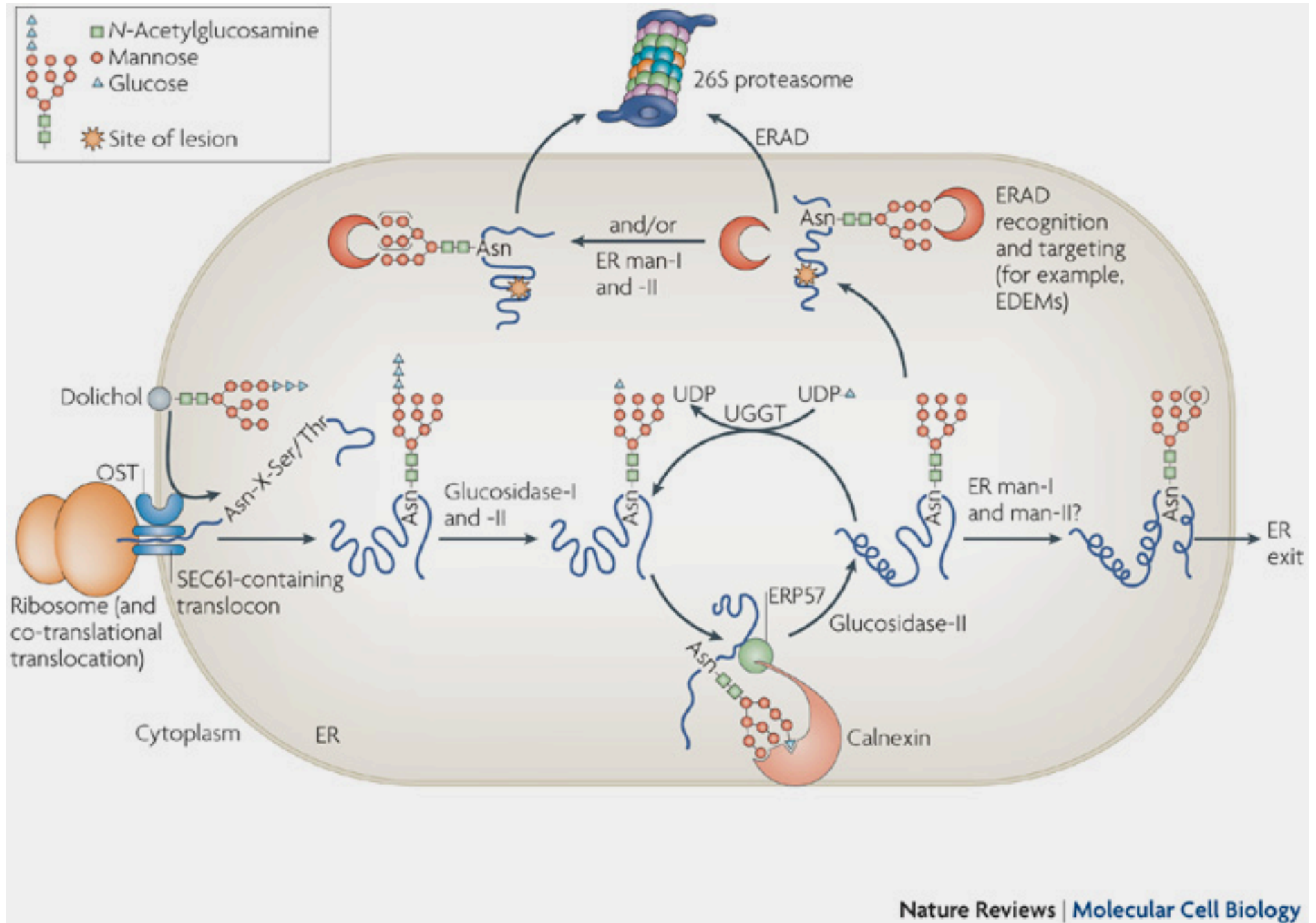


## CDG Disorders by Compartment

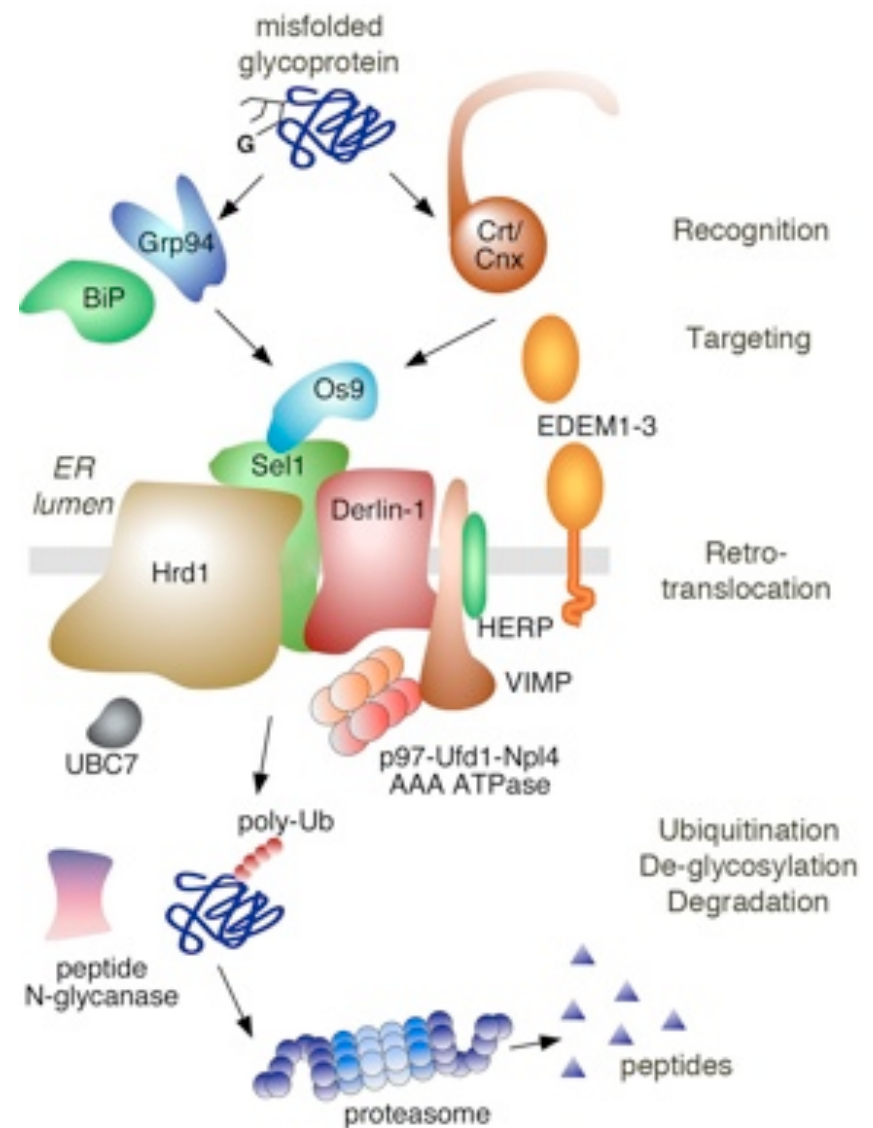
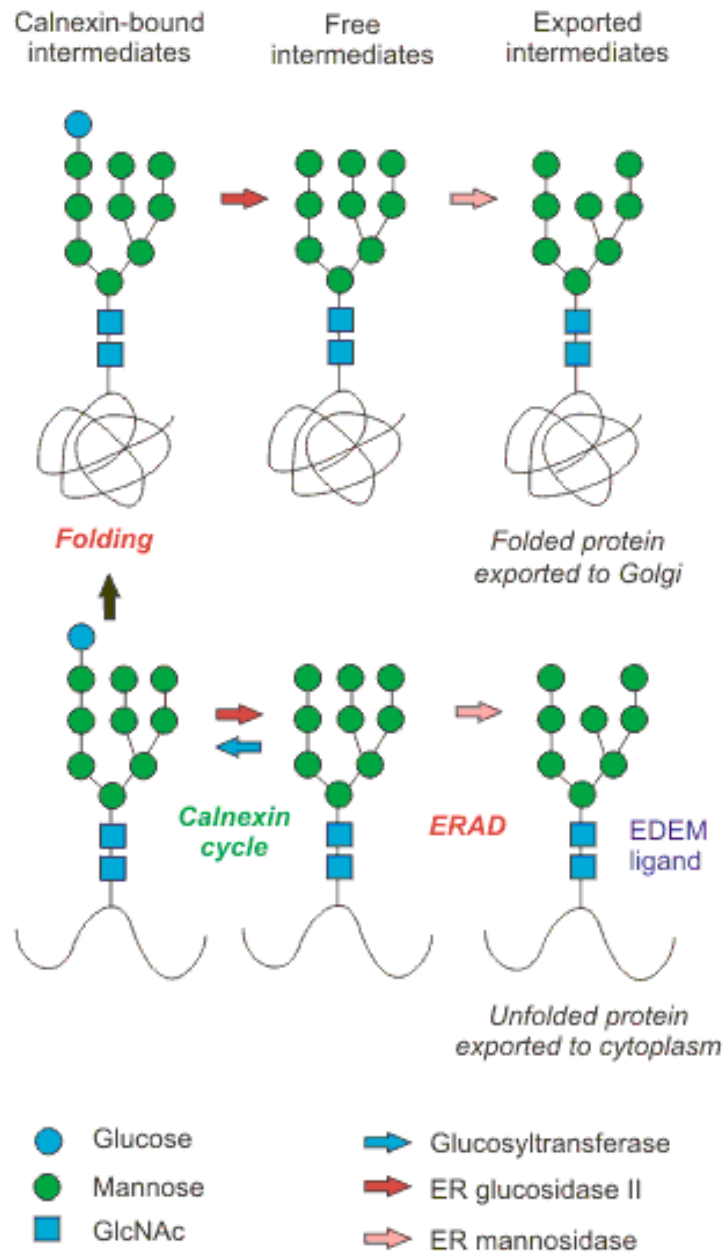


Adapted from J Inherit Metab Dis (2011) 34:853-858  
Thanks to Lynne Wolfe NP and Donna Krasnewich, MD, NGMS

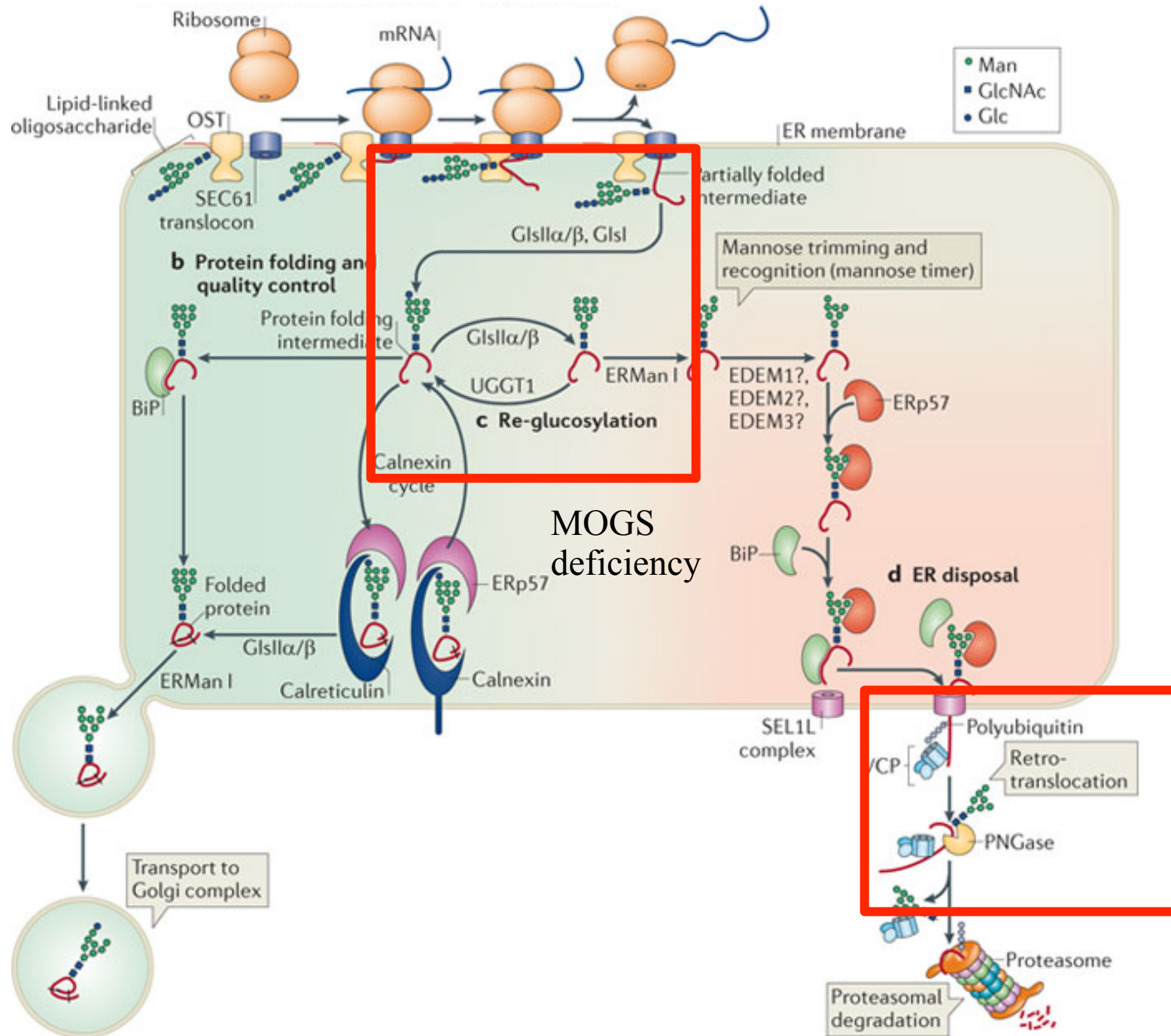
# The essential role of N-Glycosylation in ER Protein Folding



# N-Glycans in Protein Folding Cycle and ERAD



# N-Glycan Biosynthetic Pathway and ER Quality Control



MOGS deficiency

NGLY1 deficiency

MEDICAL DISPATCH | JULY 21, 2014 ISSUE

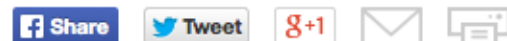
## ONE OF A KIND

*What do you do if your child has a condition that is new to science?*

BY SETH MNOOKIN



TABLE OF CONTENTS



MEDICAL DISPATCH

### ONE OF A KIND

*What do you do if your child has a condition that is new to science?*

BY SETH MNOOKIN

Matt Might and Cristina Canosa met in the spring of 2003, when the 27-year-old neurogeneticist at the Georgia Institute of Technology Cristina was an industrial-design major with an interest in philosophy. Matt was a shy computer-geek obsessed with "Star Trek." At first, Cristina took no notice of him, but the more she learned about him, the more she was drawn to him, and that fall



Unusually, the young boy was the only known patient with a rare genetic disorder. His parents began searching for others.

they began dating. Within a year, they were married. The couple had their first child, a son, on December 9, 2007, not long after Matt completed his Ph.D. in computer science and Cristina earned her M.B.A. They named him Bernard, in honor of the British physician and neuroanatomist John Bernard Russell. After a few blissful weeks, the new parents began to

decide they were unusual that his development was within normal variations. But afterwards, when Matt was able to reach his phone, he would tell Cristina he had left several messages. "I didn't know," he told her in an email. "I didn't know to the number of them told me this was really bad."

Bernard had brain damage—yes, at least, that was the diagnosis with a 90 percent chance that his brain was perfectly normal. After a new round of lab work, Matt and Cristina's doctors concluded that he likely had a non-inherited movement disorder called neurodegeneration. A subsequent genetic screen ruled out that diagnosis. When Bernard was three months old, the Might-Canosa family moved to Saly Lake, where Bernard's

inbreeding often resulted in a rare, but constant, more common condition called autism. That afternoon, when Matt was able to reach his phone, he would tell Cristina he had left several messages. "I didn't know," he told her in an email. "I didn't know to the number of them told me this was really bad."

When Bernard was a newborn, Matt asked to think that he would be as healthy as a person that he wouldn't care with a physical disability. He was just a baby, he thought. In May of 2009, the Might-Canosa family moved to Saly Lake, where Bernard's inbreeding often resulted in a rare, but constant, more common condition called autism. That afternoon, when Matt was able to reach his phone, he would tell Cristina he had left several messages. "I didn't know," he told her in an email. "I didn't know to the number of them told me this was really bad."

In September of 2012, I visited the Might-Canosa family in Saly Lake, where they live in a two-story brick Colonial-style house. Matt was a striped Brooks Brothers polo shirt and pants, with a neatly trimmed beard and shoulder-length brownish-blond hair. He brought to mind Robin Borg of the late sitcom *Seinfeld*. Cristina, who is five feet six, with porcelain skin and long black hair, greeted me with a hug and a warm smile.

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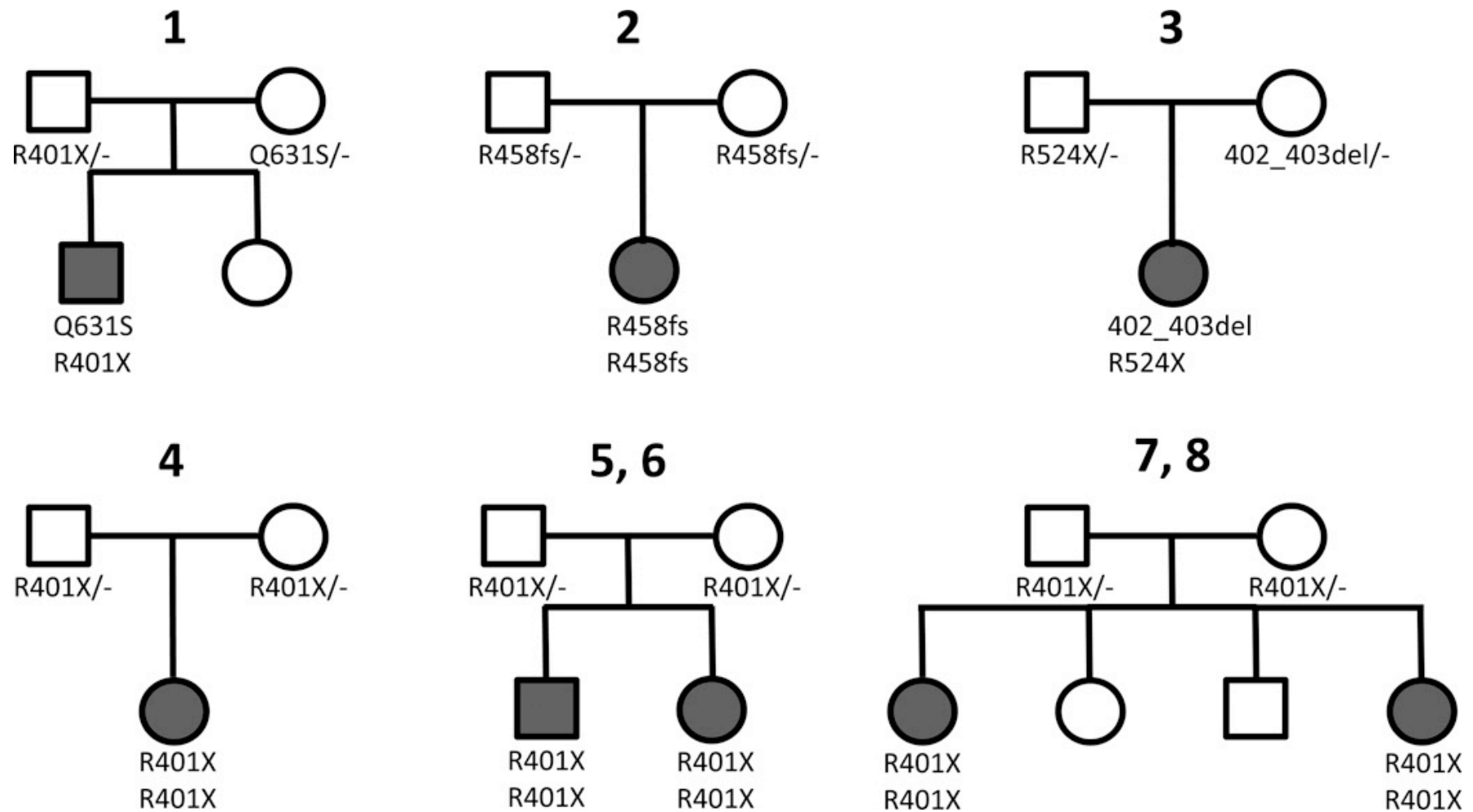
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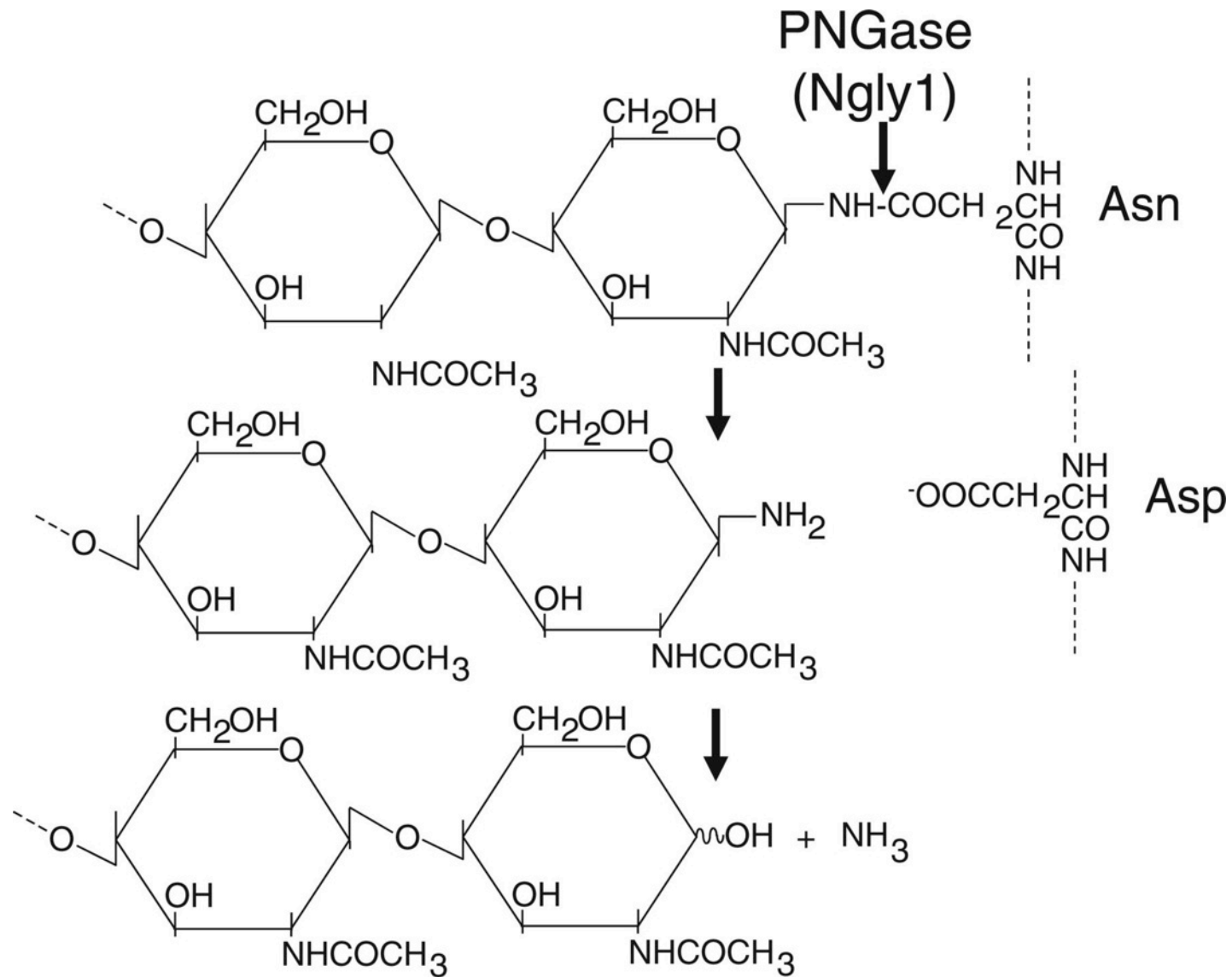


# Whole Exome Sequencing: Mutations in NGLY1 Cause an Inherited Disorder of the Endoplasmic Reticulum-Associated Degradation (ERAD) Pathway

Gregory M. Enns,...David Goldstein

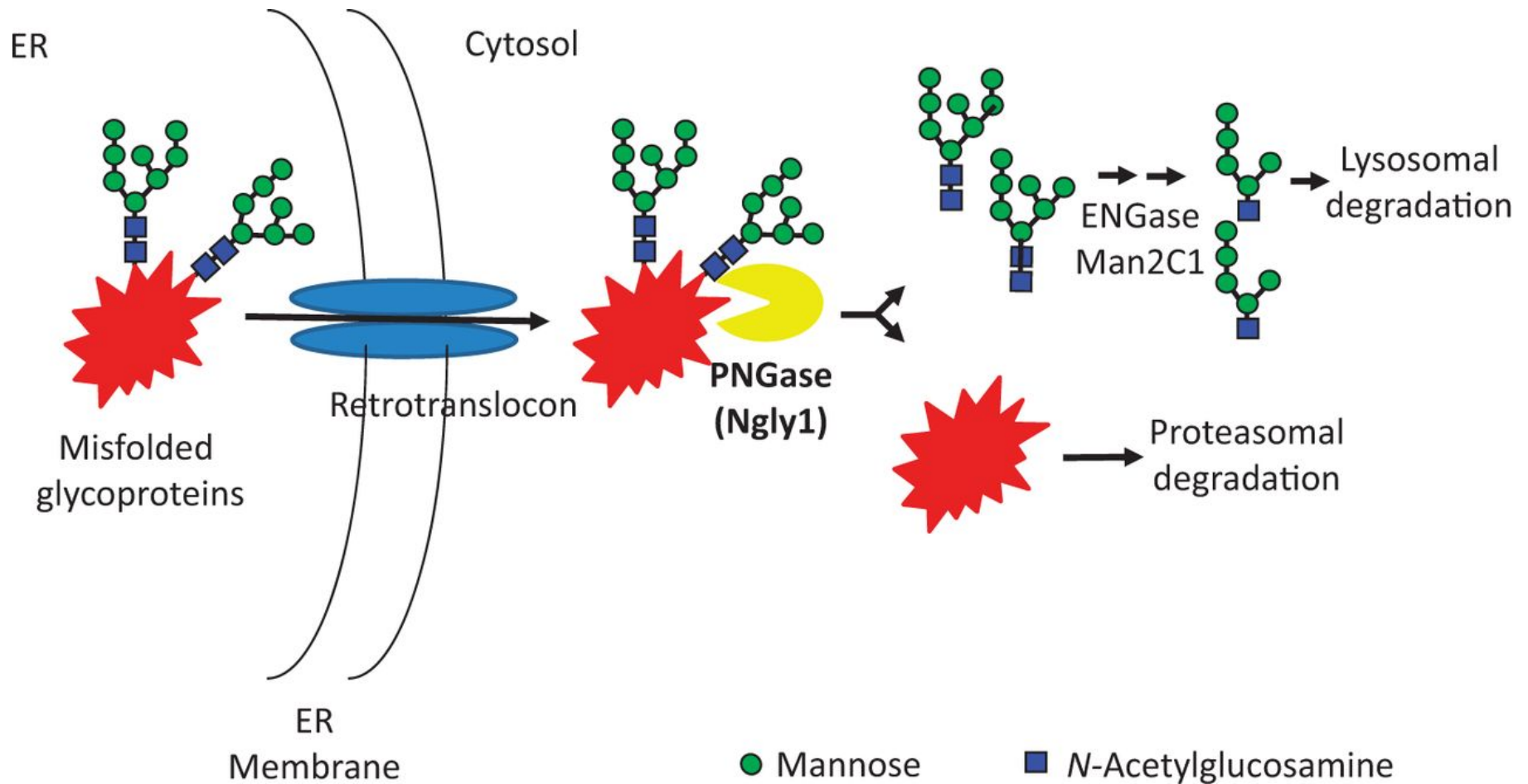


# Reaction scheme of PNGase.



Suzuki T J Biochem 2015;157:23-34

The involvement of cytoplasmic PNGase in ERAD. The glycoproteins destined for degradation are translocated from the ER lumen to the cytosol.



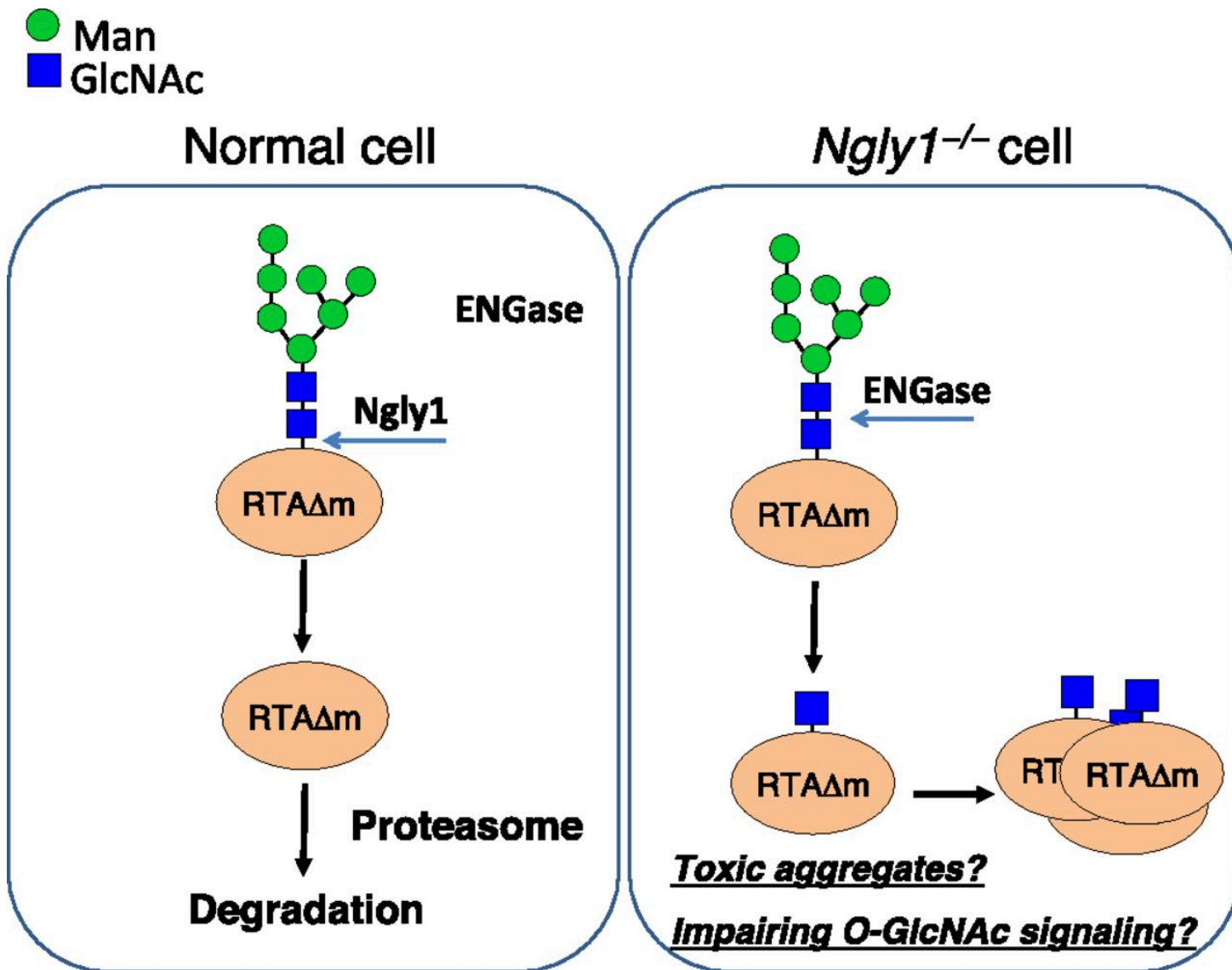
Suzuki T J Biochem 2015;157:23-34

## NGLY1 description for NGLY1.org

“We are fortunate that N-Glycanase was studied by the glycobiology community long before the discovery of the disorder.”

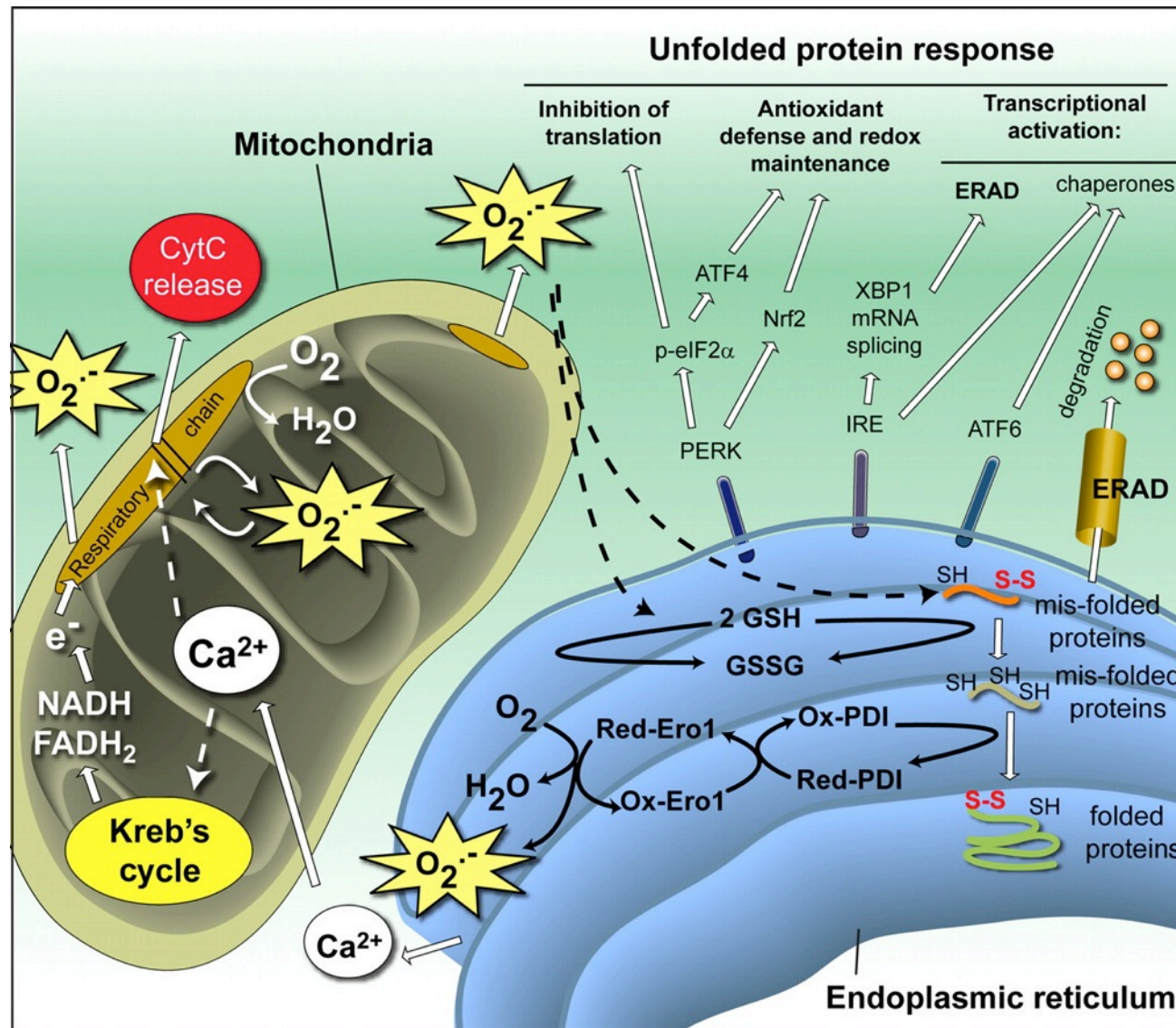
- N-Glycanase (encoded by the gene NGLY1) is responsible for cleaving N-linked glycans from misfolded glycoproteins, so that the body can recycle them.
- Lacking N-Glycanase leaves the body with an impaired capacity to recycle misfolded glycoproteins, which appear to accumulate in the cells of patients.
- The current hypothesis is that accumulation of these misfolded glycoproteins is what causes the harm in these patients.

# Schematic representation of ENGase-mediated formation of N-GlcNAc proteins in Ngly1<sup>-/-</sup> cells.



Huang C et al. PNAS 2015;112:1398-1403

# ROS generation by the ER and its interrelations to mitochondrial ROS generation.



Bashan N et al. *Physiol Rev* 2009;89:27-71

Physiological Reviews

# Clinical Features of NGLY1 deficiency

In addition to global developmental delay, neurological impairment, movement disorder and hypotonia, there are some symptoms that only appear in subgroups of the patient population.

Each of the following symptoms has been found in at least half of all patients:

- **A lack of tears**
- **Liver dysfunction:** In particular, alpha-fetoprotein (AFP) may be extremely elevated while young.
- **A smaller head** (around the 5th percentile).
- **Diminished reflexes**
- **material stored in liver cells:** There appears to be something stored in the cytoplasm of liver cells.
- **Seizures:** About half of all patients have observable seizures. Patient EEGs are often described as "abnormal."

ORIGINAL ARTICLE

# Clinical application of exome sequencing in undiagnosed genetic conditions

Anna C Need,<sup>1</sup> Vandana Shashi,<sup>2</sup> Yuki Hitomi,<sup>1</sup> Kelly Schoch,<sup>2</sup>  
Kevin V Shianna,<sup>1</sup> Marie T McDonald,<sup>2</sup> Miriam H Meisler,<sup>3</sup> David B Goldstein<sup>1,4</sup>

*J Med Genet* 2012;**49**:353–361

**Table 1** Demographic and clinical features of sequenced patients

Trio	Sex	Age	Race	Symptoms
2	M	3	European-American	Developmental delay, multifocal epilepsy, involuntary movements, abnormal liver function, absent tears

*J Med Genet* 2012;**49**:353—361

1<sup>st</sup> disorder of DE-Glycosylation

# Mutations in *NGLY1* cause an inherited disorder of the endoplasmic reticulum–associated degradation pathway

Gregory M. Enns, MB, ChB<sup>1</sup>, Vandana Shashi, MD, MBBS<sup>2</sup>, Matthew Bainbridge, PhD<sup>3</sup>, Michael J. Gambello, MD, PhD<sup>4</sup>, Farah R. Zahir, PhD<sup>5</sup>, Thomas Bast, MD<sup>6</sup>, Rebecca Crimian, MS<sup>2</sup>, Kelly Schoch, MS<sup>2</sup>, Julia Platt, MS<sup>1</sup>, Rachel Cox, MS<sup>1</sup>, Jonathan A. Bernstein, MD, PhD<sup>1</sup>, Mena Scavina, DO<sup>7</sup>, Rhonda S. Walter, MD<sup>8</sup>, Audrey Bibb, MS<sup>4</sup>, Melanie Jones, PhD<sup>4</sup>, Madhuri Hegde, PhD<sup>4</sup>, Brett H. Graham, MD, PhD<sup>3</sup>, Anna C. Need, PhD<sup>9</sup>, Angelica Oviedo, MD<sup>10</sup>, Christian P. Schaaf, MD, PhD<sup>3,11</sup>, Sean Boyle, PhD<sup>12</sup>, Atul J. Butte, MD, PhD<sup>12</sup>, Rong Chen, PhD<sup>12</sup>, Michael J. Clark, PhD<sup>12</sup>, Rajini Haraksingh, PhD<sup>12</sup>, Tina M. Cowan, PhD<sup>13</sup>, FORGE Canada Consortium, Ping He, MD, PhD<sup>14</sup>, Sylvie Langlois, MD<sup>5</sup>, Huda Y. Zoghbi, MD<sup>3,11,15</sup>, Michael Snyder, PhD<sup>12</sup>, Richard Gibbs, PhD<sup>3</sup>, Hudson H. Freeze, PhD<sup>14</sup> and David B. Goldstein, PhD<sup>16,17</sup>

# Clinical Features of NGYL1

## Found at NIH

Developmental delay (8/8)	
Movement disorder (8/8)	
Hypotonia (8/8)	
Alacrima/hypolacrima (7/8)	Hypolacrima on Schirmer testing (8/8)
EEG abnormalities (7/8)	Optic nerve pallor (6/8)
Constipation (7/8)	Mild peripheral retinal pigmentary changes (5/8)
Transaminase elevation (6/7)	Near normal peripheral hearing sensitivity (8/9)
Microcephaly (6/8)	Hyperkinetic movement disorder (9/9)
Decreased reflexes (6/8)	Abnormal sweat response in a length dependent manner (5/8)
Abnormal brain imaging (6/8)	ABR Delayed and/or dysynchronous transmission through the brainstem (7/8)
Abnormal liver storage (5/6)	History of absence/atonic/myoclonic seizures (5/9)
IUGR (5/8)	Delayed bone age (6/8)
Elevated blood lactate (4/6)	Developmental delay (9/9)
Seizures (4/8)	Demyelinating Axonal sensorimotor polyneuropathy (5/8)
Strabismus (4/8)	Mostly resolved transaminitis (6/9 (one liver transplant))
Corneal disease (4/8)	Abnormal liver texture on ultrasound (5/9)
Chalazions (4/8)	Cerebral atrophy (3/8)
Ocular apraxia (4/8)	
Neonatal jaundice (4/8)	
Dysmorphic features (4/8)	
Scoliosis (4/8)	
Small hands/feet (4/8)	
Peripheral neuropathy (3/3)	
Elevated AFP (3/5)	
ABR abnormalities (2/5)	
Liver fibrosis (2/6)	

# Clinical Features of NGYL1

## **NEW Findings from NIH**

Abnormal neurotransmitter levels (3/8)

Low CSF protein (6/8) and albumin levels (8/8)

Hyper immune response to the rubella and rubeola vaccination (7/8),

Lower than predicted resting energy expenditure (8/8)

Consistently affectionate and happy demeanor (8/8)

## **PERTINENT NEGATIVES from NIH**

Normal echocardiogram

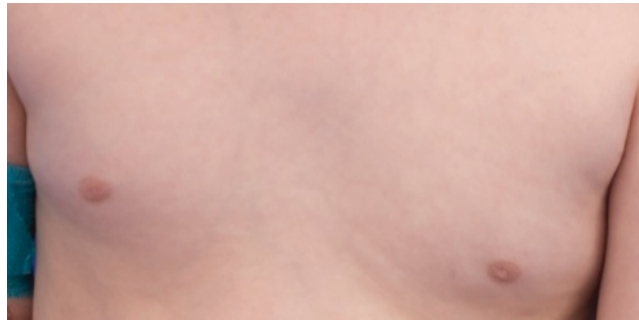
Normal gastric fluid pH

No evidence of primary muscle disease on EMG

No evidence of aspiration by Swallow study



**NGLY1-CDG**  
**NIH**



# NGLY1-CDG

## NIH

